

CLAIMS

1. An isolated, purified or recombinant nucleic acid sequence comprising:
 - (a) a sequence that encodes the apophotoprotein of pholasin (alternatively, 'apopholasin');
 - 5 (b) a sequence substantially homologous to or that hybridises to sequence (a) under stringent conditions; or
 - (c) a sequence substantially homologous to or that hybridises under stringent conditions to the sequence (a) or (b) but for the degeneracy of the genetic code; or
- 10 (d) an oligonucleotide specific for any of the sequences (a), (b) or (c)

PROVIDED THAT such homologous sequences according to (b) or (c) encode a protein capable of binding to luciferin.

2. A sequence according to claim 1, wherein the sequence that encodes for apopholasin is as shown in Figure 4B.
- 15 3. A sequence according to claim 1, wherein the sequence that encodes for apopholasin is as shown in any one of Figures 1, 2, 3, 4A, 6 or 9.
4. A sequence according to any preceding claim, wherein the apopholasin is non-glycosylated.
- 20 5. A sequence according to any preceding claim, wherein the apopholasin is glycosylated.
6. An isolated, purified or recombinant construct incorporating a sequence encoding an apophotoprotein whose expression in a substrate, in association with a luciferin therefor, signals the presence of oxygen or an oxygen metabolite in the substrate.
7. An isolated, purified or recombinant construct incorporating a sequence encoding 25 an apophotoprotein whose expression in a substrate, in association with a luciferin therefor, signals the presence of oxygen or an oxygen metabolite in the absence of a corresponding luciferase in the substrate.
8. A construct according to claim 6 or claim 7, wherein the apophotoprotein is apopholasin.
- 30 9. A recombinant construct according to any one of claims 1 to 8, wherein the nucleic acid sequence is linked operably with nucleotides enabling expression and secretion of the apopholasin in a cellular host.
10. DNA or RNA according to any of claims 1 to 9.

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11. An isolated, purified or recombinant polypeptide comprising apophotoprotein Gb 002903504 pholasin (apopholasin), or a mutant or variant thereof, which mutant or variant is capable of binding to luciferin.

12. An isolated, purified or recombinant polypeptide according to claim 11 comprising the amino acid sequence of Figure 4 or Figure 5.

13. The apopholasin according to claim 11 or claim 12 when expressed by recombinant DNA or RNA according to claim 10.

14. The apopholasin according to claim 13, which is non-glycosylated.

15. A cell, plasmid, virus or live organism having incorporated expressibly therein a sequence according to any one of claims 1 to 10, whereby it is capable of producing an apoprotein.

16. A vector comprising a sequence according to any one of claims 1 to 10.

17. A host cell transformed or transfected with a vector according to claim 16.

18. A bioluminescent oxidative indicator protein (BOIP), comprising an apophotoprotein according to any one of claims 11 to 14 in association with a luciferin.

19. A BOIP according to claim 18, wherein the luciferin is derived from *Pholas dactylus*.

20. A method for the preparation of a bioluminescent oxidative indicator protein (BOIP), which method comprises bringing an apophotoprotein into association with a luciferin therefor.

21. A method for the detection and/or measurement of oxygen or one of its metabolites extracellularly, which method comprises providing a bioluminescent oxidative indicator protein (BOIP) extracellularly and thereafter detecting and/or quantifying light emission therefrom and/or changes in colour, intensity and/or polarisation of emission(s), wherein the apophotoprotein comprises recombinant apopholasin.

22. A method for the detection and/or measurement of oxygen or one of its metabolites in live cells (intracellularly), which method comprises providing a BOIP intracellularly and thereafter detecting and/or quantifying light emission therefrom and/or changes in colour, intensity and/or polarisation of emission(s) therefrom.

23. A method according to claim 21 or 22, wherein said BOIP is selected from native or chemically- or genetically- modified BOIP or a 'rainbow protein' based on such a BOIP.

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24. A method according to any one of claims 21 to 23, wherein said BOP includes a signal peptide, targetting it to a pre-determined extra- or intra-cellular site.

25. A method according to any one of claims 21 to 23, comprising incubating a test sample with a cell according to claim 15 or with a membrane preparation derived therefrom.

5 26. A method according to any one of claims 21 to 24, wherein light emission takes place in the absence of a luciferase.

27. The use of a sequence or a protein according to any one of claims 1 to 19 in the detection, diagnosis or measurement of oxygen or a metabolite thereof.

10 28. A diagnostic kit incorporating a sequence or protein according to any one of claims 1 to 19.

29. A method for obtaining a substantially homologous source of apopholasin, which method comprises culturing cells having incorporated expressibly therein a polynucleotide encoding apopholasin as defined in any one of claims 1 to 10, and thereafter recovering the cultured cells.

15 30. A method, use or kit according to any one of claims 20 to 29, substantially as hereinbefore described with particular reference to the Examples.